THE "HERTWIG EFFECT" IN TELEOST DEVELOPMENT

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In 1911 O. Hertwig found that when frog sperm were treated to prolonged exposures of radium they retained their ability to fertilize eggs but lost their genetic function. The result was similar to parthenogenetic (gynogenetic) development, wherein the egg developed without benefit of sperm chromatin. Since 1911 the study of parthenogenesis produced by this method has been limited, among the vertebrates, almost exclusively to the Amphibia. It is therefore of interest to determine whether the exposure of other vertebrate sperm to ionizing radiations could similarly result in parthenogenetic development.

Materials and Method

Fundulus heteroclitus is a marine teleost common in the Woods Hole area, readily obtained by the Marine Biological Laboratory Supply Department. They are kept in the laboratory in running sea water until used. The method for obtaining eggs and rearing the embryos is that described by Costello *et al.* (1957).

Prior to x-irradiation a concentrated suspension of sperm was prepared by removing the testes from five to six sexually mature males and placing them on a plastic depression plate and macerating them. A portion of this suspension was then removed to another and similar plate to be kept and used as control. The remaining sperm were irradiated in a cesium-137 irradiator at an output of 5000 r/min. The exposures used were 500 r, 1000 r, 5000 r, 50,000 r, 100,000 r, and 150,000 r. Sperm samples were removed at appropriate intervals for the fertilization of normal eggs.

Fertilization was accomplished by placing normal eggs, recently stripped, in a stender dish with a very small volume of filtered sea water, and adding the sperm by means of a glass rod dipped into the appropriate suspension. Between each use the rod was washed in tap water to kill any adherent sperm, and was thoroughly dried. In the earlier series the fertilization occurred immediately after irradiation of each portion of sperm, but since the time required for the highest level of irradiation was brief, all later series were fertilized simultaneously following the completion of all irradiations. It was found that sperm samples added to the normal eggs were all active, showing motility even after 150,000 r.

The developing embryos were raised in fingerbowls containing filtered sea water, to avoid unnecessary contamination. All non-cleaving eggs were removed after the cleaving eggs had attained the blastodisc stage. At the end of the experiment the embryos were again photographed, and fixed in Bouin's solution and

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prepared for possible cytological study according to the method of Costello *et al.* (1957).

Experimental Data

In all cases, the per cent of eggs which cleaved, following fertilization with x-irradiated sperm, was half or less than that of the control. The per cent cleavage did not necessarily decrease proportionately with increased exposure to the sperm (see Table I). Normal variations in cleavage per cent are such that separate controls of the same eggs were used for each series. As the season progressed fewer eggs were available and fewer of the control eggs developed.

Among those eggs fertilized with irradiated sperm there was no observable lag in the cleavage time for the first division and until the blastula stage, when compared with the controls. This held true for all irradiation levels, even at 150,000 r to the sperm. Following gastrulation (stage #12; see Oppenheimer, 1937; Solberg, 1938; or Rugh, 1962) most eggs fertilized by sperm which had

Table I

*Percentage of eggs developing (cleaving) after fertilization with x-irradiated sperm**

Exposure (r)	Series 1	Series 2	Series 3
Control–0	84.1 (107)	100 (25)	20 (5)
500 r	29.5 (44)		
1000 r	47.2 (36)		
2000 r	42.0 (50)		
5000 r	47.1 (34)	46.6 (30)	
50,000 r	53.1 (49)	7.1 (28)	20 (10)
100,000 r		3.4 (29)	7.7 (13)
150,000 r			7.1 (14)

* Note: Total number of eggs examined in parentheses.

been exposed to 5000 r or more showed some slight retardation over the controls, to the extent of about one full stage of development (Plate I, Figs. 1–8). Those eggs fertilized with sperm receiving the higher exposures of 50,000 r or more showed a slightly greater retardation than those fertilized by sperm which had been irradiated to a lower level. However, the anomalies following fertilization with 5000 r sperm appeared to be more severe than those arising from sperm exposed to higher levels of irradiation. Some retardation was seen in eggs fertilized by 2000 r sperm, and this retardation occurred beginning at stage #14, while the lower exposures delayed the retardation to stage #22. Sperm exposed to 500 r were unable to adversely affect development.

In eggs fertilized by sperm exposed to 5000 r or 50,000 r, some appeared to develop equally well with the controls in every respect (Plate I, Figs. 9, 10 and Plate II, Figs. 11, 12).

More than half of the embryos developing from eggs fertilized by sperm exposed to 5000 r or more developed pulsating hearts and pigment patterns similar to those of the controls, but all were stunted or otherwise malformed with the exception of the few aforementioned (Plate I, Figs. 5–8, Plate II, Figs. 13–16). Some of these embryos developed corpuscles, and many showed these corpuscles

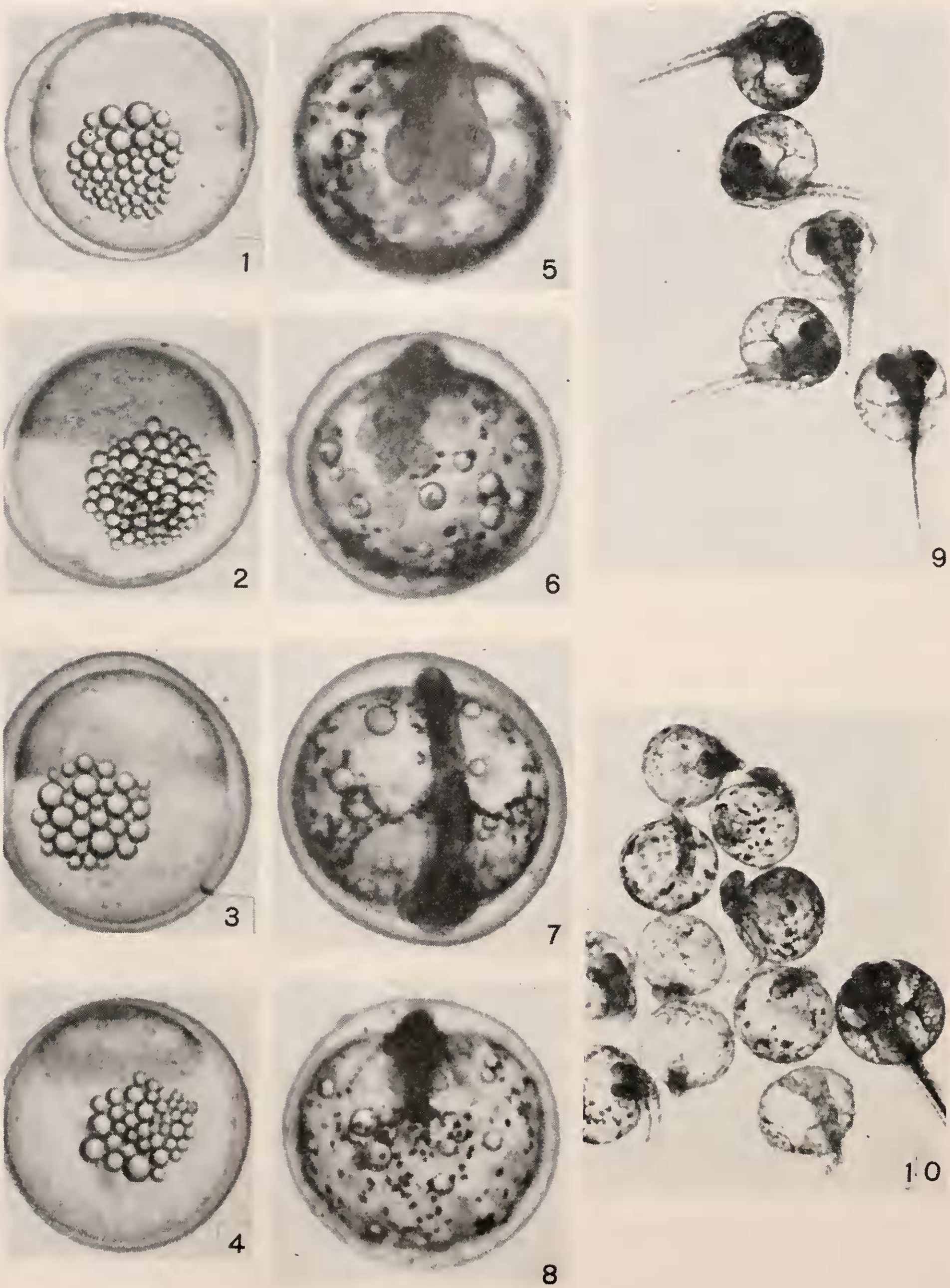

PLATE I

FIGURES 1–4. All embryos are from the same series and all are one day in development. FIGURE 1. Control, midgastrula. FIGURE 2. From 50,000 r to sperm, early gastrula. FIGURE 3. From 100,000 r to sperm, early gastrula. FIGURE 4. From 150,000 r to sperm, late blastula.

circulating. In all embryos not possessing a pulsating heart, edema developed. The heart beat, even without corpuscles, was found to be of a rate similar to that of the normal controls with their full complement of corpuscles.

One severe abnormality rather common to both the 5000 r and the 50,000 r series was the failure of the embryo to form either a neural or body axis. The blastoderm developed into an amorphous mass of protoplasm devoid of any recognizable structure, but often possessing pigment cells on the surface. Some eggs ceased development at late blastula or early gastrula stages.

Discussion

The work of O. Hertwig (1911), Oppermann (1913), Porter (1939), Rugh (1939) and others has shown that haploid development (either androgenetic or gynogenetic) generally exhibits a specific set of anomalies. Among the Amphibia, haploid development appears to be normal until gastrulation, when a delay in development is noted when comparisons are made with simultaneous controls. Neurulation is even more delayed and abnormal. In older embryos (six to eight days), the brain is poorly differentiated and the circulatory system is non-functional, although heart and corpuscles may have formed. Edema generally appears under such conditions, probably because of the failure in excretory function. Tail formation is retarded, giving the haploids a stunted appearance, accentuated by lordosis. Similar observations have been made by Oppermann (1913) on parthenogenetic trout.

Some of the eggs developing from eggs fertilized by sperm exposed to 50,000 r and all embryos from sperm exposed to higher levels of irradiation showed the same characteristics described above. Since Rugh (1939) found that among the Amphibia, some 90% of the embryos developing from eggs fertilized by sperm exposed to 50,000 r were haploids, it is quite probable that the *Fundulus* embryos which survived the high levels of irradiation and appeared normal were indeed haploids and parthenogenetic at the beginning. This is supported by the fact that the eggs fertilized by sperm which had been exposed to 5000 r were more severely abnormal than any of those which survived fertilization by sperm exposed to either 100,000 r or 150,000 r. It thus appears that exposures of sperm up to 50,000 r are not quite sufficient to completely eliminate the genetic contribution of the sperm in every case.

There was one embryo from the 5000 r series and two from the 50,000 series which were indistinguishable from the controls. These were probably recovered diploid embryos. Tyler (1941) discussed the possible methods of regulation from haploidy to diploidy in the frog. Since there was no evidence of cleavage

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FIGURES 5–8. The same series of embryos seen in Figures 1–4 but 3¼ days after fertilization, each derived from the egg shown to its left. Embryo in Figure 7 is almost normal, possessing circulation comparable to the control. Note poorly formed central nervous system in all but the controls.

FIGURE 9. A group of control eggs 7 days after fertilization. Note uniformity in development.

FIGURE 10. A comparable group of embryos developing from eggs fertilized by sperm exposed to 5000 r x-rays (Cs-137), showing optimum development among this group. One embryo appears to closely resemble the controls.

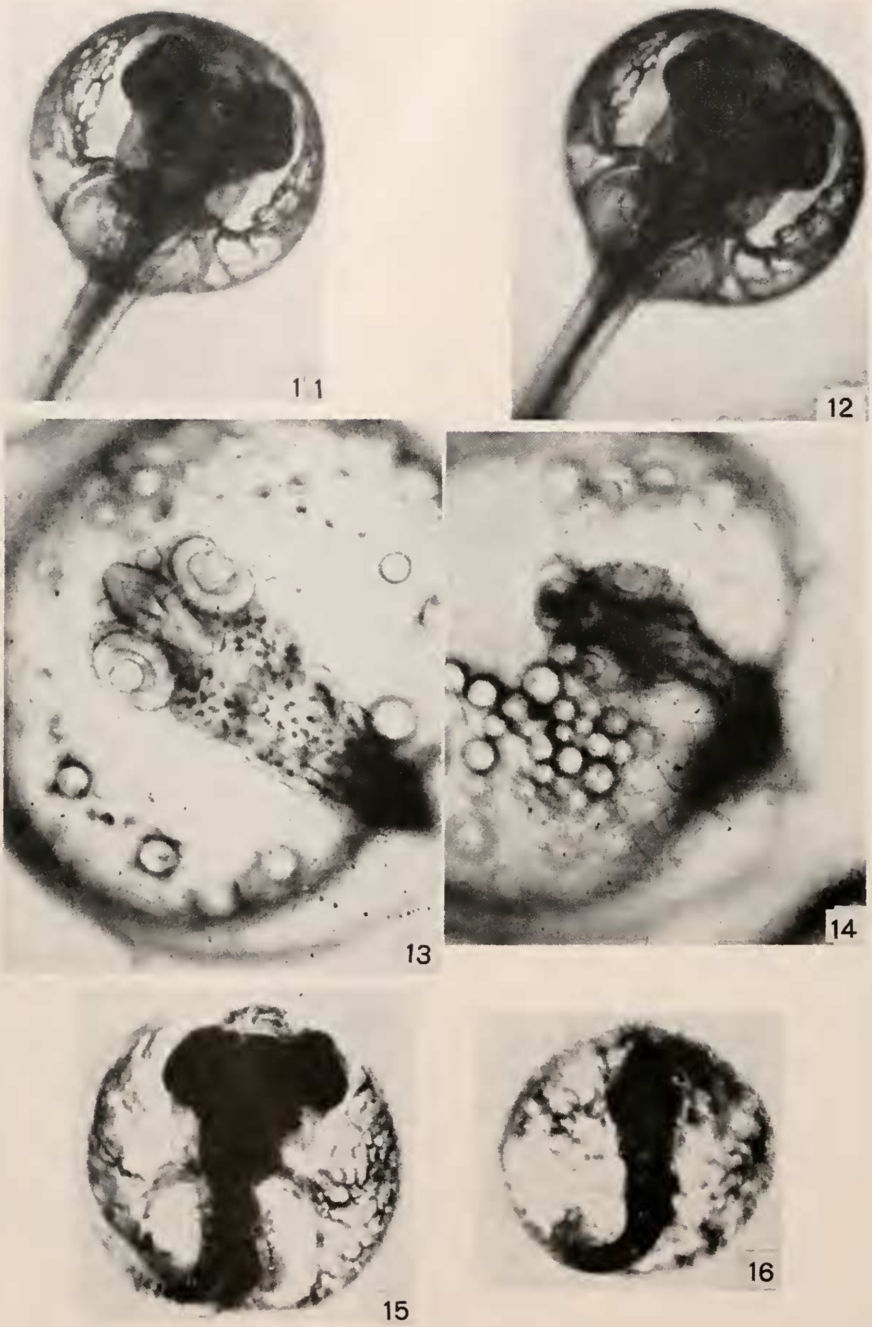

PLATE II

delay in these experiments, the other possible explanations might be considered: (1) Retention of the second polar body; (2) omission of polar divisions; (3) diploidy of virgin eggs; and (4) progressive regulation during cleavage. Information is not available as to the state of the nucleus of *Fundulus* at the time of insemination, and it is unlikely that 3 out of 42 eggs would be diploid before fertilization. Parthenogenetic development in *Fundulus* is most likely to occur from progressive regulation in eggs fertilized by sperm whose genetic complement has been destroyed by 100,000 r or more.

Many of the embryos in these experiments were able to survive for several days, even with poorly developed central nervous system and circulation. The heart was seen pulsating in some embryos even after seven days' development, and some showed muscular movements of the body and tail. It has been found that in haploid amphibian cells the utilization of yolk is much slower than in the controls (Porter, 1939). This might also be correlated with the retardation of carbohydrate metabolism. The amount of oxygen required by haploid cells is not as great as that of diploid cells, enabling them to survive for an extended period without a circulatory supply of oxygen.

A cytological study of control and experimental embryos was made in order to determine whether the parthenogenetic individuals were haploid or diploid, and whether any of their organs reflected these variables. The cells of the gut and kidney were most suitable for counting nucleoli, and the eye for organ development.

The cells of the control embryos in every case possessed paired nucleoli while the majority of the parthenogenetic embryos, from sperm exposed to 50,000 r or more, had single nucleoli. The exception was the embryo from 50,000 r sperm shown in Figure 12, which had two nucleoli per cell. This embryo could not be distinguished from the parallel control, with regard to organ differentiation and development, so that it is presumed to be a recovered-diploid embryo. The chromosomes of this form are almost impossible to count, they are so small and numerous. Many of the embryos from sperm exposed to less than 50,000 r were also probably diploid, possessing two nucleoli in each cell. Thus, such cytological study as was possible corroborated the gross findings of the "Hertwig Effect" even with the fish embryos.

It is of interest that mature sperm of *Fundulus* can tolerate exposures of 150,000 r without impairing their motility or their ability to activate normal eggs.

FIGURE 11. Control embryo at 7 days' development.

FIGURE 12. One of two embryos developing from eggs fertilized with sperm which had been exposed to 50,000 r, shown at 7 days and to be compared with that in Figure 11. Probably diploid.

FIGURE 13. Control embryo at three days of development.

FIGURE 14. An embryo developing from an egg fertilized with sperm which had been exposed to 100,000 r, now seen at three days of age, to be compared with control in Figure 13. Note malformation of brain vesicles, general retardation including the eyes.

FIGURES 15–16. The same embryos (Figures 13, 14) seen at 7 days of development. The experimental (from irradiated sperm) embryo in Figure 16 now possessing circulation. Note poorly developed nervous system, myotomes, and tail, indicating general retardation in development.

The eggs of *Fundulus* are irrevocably damaged so that they cannot develop beyond the stage #15 if they are exposed to as little as 4000 r x-rays.

Summary and Conclusions

1. Mature sperm of *Fundulus heteroclitus* were exposed to ionizing radiations from Cs.-137 at the rate of 5000 r/min., for doses ranging from 5000 r to 150,000 r, and were then used to fertilize normal eggs of the same species. Control eggs from the same batch were inseminated with unirradiated sperm from the same source.

2. While variations in normal fertilizability of the control eggs do occur, associated with the season and breeding activity, in every case some eggs were fertilized and developed following insemination with sperm which had been exposed to every dose level. This was not mere activation since cleavages followed.

3. The presence of irradiated (sperm) chromatin had no effect on the time or nature of the early cleavages. The initial adverse effects were noted at the time of gastrulation.

4. Exposures of sperm to 500 r had no apparent effect on the development of eggs, while exposures to 5000 r caused high mortality and morbidity, and above 5000 r (to the sperm) the effect of ionizing radiations appeared to decrease so that a greater percentage of near-normal embryos resulted from cleaving eggs.

5. The fact that a few specimens from 50,000 r or more sperm could not be readily distinguished from the controls suggests that complete recovery of diploidy may sometimes occur after activation. This was substantiated by cytological examination.

6. While parthenogenesis does not, or need not, occur naturally for this species, the fact that it can occur is of biological significance, suggesting that all vertebrates may possess eggs with such regulatory potentialities.

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